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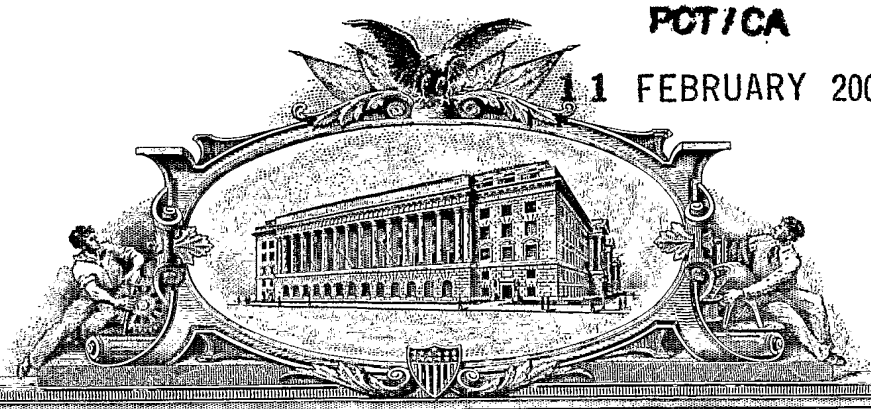

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APPLICATION NUMBER: ***60/531,605***
FILING DATE: ***December 23, 2003***

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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No.

INVENTOR(S)		
Given Name (first and middle [if any])	Family Name or Surname	Residence (City and either State or Foreign Country)
Albert Ahmad	Friesen Khalil	Winnipeg, Canada Winnipeg, Canada

Additional inventors are being named on the _____ *separately numbered sheets attached hereto*

TITLE OF THE INVENTION (500 characters max)

COMBINATION THERAPIES EMPLOYING HMG COA REDUCTASE INHIBITORS

Direct all correspondence to: **CORRESPONDENCE ADDRESS**

☐ Customer Number:

OR

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ENCLOSED APPLICATION PARTS *(check all that apply)*

☑ Specification *Number of Pages* 35 ☐ CD(s), Number _____

☑ Drawing(s) *Number of Sheets* 12 ☑ Other (specify) Abstract

☐ Application Date Sheet. See 37 CFR 1.76

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Respectfully submitted,

SIGNATURE _____

TYPED or PRINTED NAME David J. Heller

TELEPHONE 416-868-1482

Date December 22, 2003

REGISTRATION NO. 43,384 *(if appropriate)*

Docket Number: 29399-0061

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December 22, 2003

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Dear Sirs:

Re: New U.S. Provisional Patent Application
Title: COMBINATION THERAPIES EMPLOYING HMG COA REDUCTASE INHIBITORS
Inventors: Albert Friesen and Ahmad Khalil
Our File: 29399-0061

Enclosed herewith is a provisional patent application for filing in the names Albert Friesen and Ahmad Khalil.

We enclose the following documents:

Provisional Application for Patent Cover Sheet
Specification – 35 Pages
Abstract - 1 Page
Drawings - 12 Pages
Fee - $160.00 (by deposit account)

Please deduct the requisite fee in the amount of $160.00 from our Deposit Account No. 13-2400. If any further fee is due, please also deduct it from our Deposit Account.

Yours very truly,

ALBERT FRIESEN AND AHMAD KHALIL

By: _____
David J. Heller, Reg. No. 43,384

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TITLE OF THE INVENTION

[0001] COMBINATION THERAPIES EMPLOYING HMG COA REDUCTASE INHIBITORS

FIELD OF INVENTION

[0002] This invention generally relates to combination therapies employing HMG Co A reductase inhibitors and uses thereof.

BACKGROUND

[0003] According to the American Heart Association in 2000, 39.4 % of deaths were from cardiovascular disease. The risk of developing heart disease and indirectly stroke, increases steadily as blood cholesterol values rise. Elevated blood cholesterol levels are also associated with an increased risk of developing diabetes. The desirable blood levels are < 200 mg/dL. Borderline acceptable levels are in the range of 200-239 mg/dL and high risk begins at 240 md/dL or greater. It is estimated that some 102.3 million Americans have high cholesterol numbers.

[0004] Hypercholesterolemia is known to affect the responsiveness of various blood vessels to endogenous and exogenous vasoactive agents. Of particular interest is the increased responsiveness to vasoconstrictors, e.g. 5-hydroxy tryptamine and noradrenaline, and the decreased reactivity towards vasodilators, e.g. acetylcholine and nitric oxide. This together with the development of arteriosclerosis plays an important role in the progression of many cardiovascular-related disorders, such as hypertension, stroke and coronary artery disease.

[0005] Presently hypercholesterolemia is treated primarily with lipid lowering drugs such as statins, bile salt sequestrants, fibrates or niacin. While statins are arguable the most effective lipid lowering drugs available, the use of statins in combination with other drugs, such as protease inhibitors (e.g. norvir), acetaminophen,cyclosporine, mibefradil, azole fungicides, macrolide antibiotics, and warfarin, is limited due to adverse drug-drug reactions, including most significantly,

the inhibition of hepatic cytochrome P450 enzymes, which are responsible for the metabolism of drugs in the liver.

[0006] In contrast, vitamin B6 which also has lipid lowering properties, is a well tolerated drug with no significant side effects (Brattstrom et al, Pyroxidine reduces cholesterol and low-density lipoprotein and increases antithrombin III activity in 80 year old men with low plasma pyridoxal 5-phosphate, Scand J Clin Lab Invest, 1990, 50:873). Several vitamin B6 derivatives also have lipid-lowering properties. For example, US Patent No. 6,066,659 teaches the use of vitamin B6 (pyridoxine), pyridoxal and pyridoxamine derivatives for the treatment of hyperlipidemia and atherosclerosis. German Patent DE 24 61 742 C2 teaches the use of pyridoxal, pyridoxol, and pyridoxamine -5'phosphoric acid esters for treating hyperlipidemia. Supplementation with magnesium pyridoxal-5'-phosphate glutamate, has also been shown to reduce lipid levels (Khayyal et al, Effect of magnesium pyridoxal 5-phosphate glutamate on vascular reactivity in experimental hypercholesterolemia, Drugs Exp Clin Res. 1998, 24:29-40).

[0007] In addition to lipid lowering properties, vitamin B6 and its metabolites, such as pyridoxal-5'-phosphate, are useful in the treatment of cardiovascular or related disease, for example, myocardial ischemia and ischemia reperfusion injury, myocardial infarction, cardiac hypertrophy, hypertension, congestive heart failure, heart failure subsequent to myocardial infarction, vascular disease including atheroclerosis, and diseases that arise from thrombotic and prothrombotic states in which the coagulation cascade is activated.

[0008] Previous disclosures have taught the optional use of vitamin B6 (pyroxidine) with a cholesterol-lowering agent wherein the inclusion of vitamin B6 was directed to decreasing homocysteine levels. For example, US Patent 6,576,256 discloses a method of treating a patient with elevated cardiovascular risk by the use of a HMG CoA reductase inhibitor with an inhibitor for the renin-angiotension system, aspirin and optionally vitamin B6 (pyridoxine). US Patent

Application No. 20030049314 discloses a formulation for treating a patient with elevated cardiovascular risk comprising a combination of an HMG Co A reductase inhibitor, an ACE inhibitor, aspirin and optionally vitamin B6. US Patent Application No. 20030068399 discloses an orally administrable pharmaceutical dosage form for treating a patient at elevated cardiovascular risk comprising a combination of comprising a combination of an HMG Co A reductase inhibitor, an inhibitor for the renin-angiotension system, aspirin and optionally vitamin B6. However, there are currently no combination therapies which employ a vitamin B6 related compound as a lipid-lowering agent in combination with a HMG Co A reductase inhibitor.

[0009] The use of statins in combination with other drugs, and consequently the potential for additive therapeutic benefits, has been limited because of hepatotoxicity. There are currently no combination therapies for treating and preventing hypercholesterolemia and related disorders such as cardiovascular disease and diabetes which do not induce adverse drug reactions and which are suitable for persons susceptible to drug-induced heptotoxicity. Accordingly, there is a need for new pharmaceutical compositions and methods of treatment which overcome the limitations of the current therapies involving statins.

SUMMARY OF INVENTION

[0010] The present invention provides a pharmaceutical composition comprising: (a) a HMG CoA reductase inhibitor; (b) a vitamin B6 related compound; and (c) a pharmaceutically acceptable carrier.

[0011] In one embodiment of the invention, the HMG CoA reductase inhibitor is selected from a group consisting: pravatstatin, lovastatin, fluvastatin, atorvastatin, simvastatin, rosuvastatin, velostatin, fluindostatin, and a mixture thereof.

[0012] In another embodiment of the invention, the vitamin B6 related compound is selected from a group consisting: pyridoxal, pyridoxal-5'-phosphate, pyridoxamine,

a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, and a mixture thereof.

[0013] The present invention also provides a method for treating a patient at risk of cardiovascular disease comprising administering a therapeutically effective dose of the pharmaceutical composition comprising: (a) a HMG CoA reductase inhibitor; (b) a vitamin B6 related compound; and (c) a pharmaceutically acceptable carrier.

[0014] In an embodiment, the method is for treating a patient at risk of cardiovascular disease. In another embodiment, the method is for treating the patient susceptible to hepatotoxicity.

[0015] The cardiovascular disease may be selected from a group consisting: congestive heart failure, myocardial ischemia, arrhythmia, myocardial infarction, ischemic stroke, hemorrhagic stroke, coronary artery disease, hypertension (high blood pressure), atherosclerosis (clogging of the arteries), aneurysm, peripheral artery disease (PAD), thrombophlebitis (vein inflammation), diseases of the heart lining, diseases of the heart muscle, carditis, congestive heart failure, endocarditis, ischemic heart disease, valvular heart disease (malfunction of a valve or valves in the blood vessels of the heart), arteriosclerosis (hardening of the arteries), acute coronary syndrome (ACS), deep vein thrombosis (DVT), Kawazaki disease, and heart transplant.

[0016] The present invention also provides a method for treating a patient at risk of diabetes comprising administering a therapeutically effective dose of the pharmaceutical composition comprising: (a) a HMG CoA reductase inhibitor; (b) a vitamin B6 related compound; and (c) a pharmaceutically acceptable carrier.

[0017] The present invention also provides a method for treating a patient at risk of Alzheimer's disease comprising administering a therapeutically effective dose of

the pharmaceutical composition comprising: (a) a HMG CoA reductase inhibitor; (b) a vitamin B6 related compound; and (c) a pharmaceutically acceptable carrier.

[0018] The present invention also provides a method for treating a patient at risk of osteoporosis comprising administering a therapeutically effective dose of the pharmaceutical composition comprising: (a) a HMG CoA reductase inhibitor; (b) a vitamin B6 related compound; and (c) a pharmaceutically acceptable carrier.

[0019] The dose of the HMG CoA reductase inhibitor may be between 0.1 and 1000 mg per day. The dose may be 10 mg per day.

[0020] The dose of the vitamin B6 related compound may be between 0.1 to 50 mg/kg per day. The dose of vitamin B6 related compound may be between 1 to 15 mg/kg per day.

[0021] The present invention further provides a method of treating or preventing hypercholesterolemia in a patient comprising administering a therapeutically effective dose of a vitamin B6 related compound wherein the vitamin B6 related compound is selected from a group consisting: pyridoxal-5'-phosphate, a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, and a mixture thereof.

BRIEF DESCRIPTION OF THE FIGURES

[0022] Figure 1 comprises line graphs 1(a) to 1(f), illustrating the decrease in the fluorescence of the metabolic products (CHC, 7-HC, HFC, fluorescein, AHMC and quinolinol) measured in the CYP inhibition assays as a function of pyridoxal 5'-phosphate concentration.

[0023] Figure 2 comprises line graphs 2(a) and 2(b) illustrating the inhibition of the catalytic activity of CYP1A2 (metabolism of CEC to CHC) as a function of Furafylline and P5P concentration respectively.

[0024] Figure 3 comprises line graphs 3(a) and 3(b) illustrating the inhibition of the catalytic activity of CYP2A6 (metabolism of coumarin to 7-HC) as a function of Tranylcypromine and P5P concentration respectively.

[0025] Figure 4 comprises line graphs 4(a) and 4(b) illustrating the inhibition of the catalytic activity of CYP2B6 (metabolism of EFC to HFC) as a function of Tranylcypromine and P5P concentration respectively.

[0026] Figure 5 comprises line graphs 5(a) and 5(b) illustrating the inhibition of the catalytic activity of CYP2C8 (metabolism of DBF to Fluorescein) as a function of Quercetin and P5P concentration respectively.

[0027] Figure 6 comprises line graphs 6(a) and 6(b) illustrating the inhibition of the catalytic activity of CYP2C9 (metabolism of MFC to HFC) as a function of Sulfaphenazole and P5P concentration respectively.

[0028] Figure 7 comprise line graphs illustrating 7(a) and 7(b) the inhibition of catalytic activity of CYP2C19 (metabolism of CEC to CHC) as a function of Tranylcypromine and P5P concentration respectively.

[0029] Figure 8 comprises line graphs 8(a) and 8(b) illustrating the inhibition of the catalytic activity of CYP2D6 (metabolism of AMMC to AHMC) as a function of Quinidine and P5P concentration respectively.

[0030] Figure 9 comprises line graphs 9(a) and 9(b) illustrating the inhibition of the catalytic activity of CYP2E1 (metabolism of MFC to HFC) as a function of Diethyldithiocarbamic acid (DDTC) and P5P concentration respectively.

[0031] Figure 10 comprises line graphs 10(a) and 10(b) illustrating the inhibition of the catalytic activity of CYP3A4 (metabolism of BFC to HFC) as a function of Ketoconazole and P5P concentration respectively.

[0032] Figure 11 comprises line graphs 11(a) and 11(b) illustrating the inhibition of the catalytic activity of CYP3A4 (metabolism of BQ to Quinolinol) as a function of Ketoconazole and P5P concentration.

[0033] Figure 12 is a table summarizing the IC_{50} values estimated for the known inhibitors of each CYP subtype, and for pyridoxal 5'-phosphate.

DETAILED DESCRIPTION OF THE INVENTION

[0034] The causal association between elevated low density lipoproteins (LDL) levels and the risk for developing cardiovascular disease is well established. Reducing elevated LDL levels have been shown to reduce the incidence of cardiovascular events, including transient ischemic attacks and indirectly strokes, and to reduce mortality. More recently, elevated LDL levels have been correlated with an increased risk for developing diabetes. The inventors of the present invention have discovered that statins and vitamin B6 related compounds in combination reduce the risk of cardiovascular disease and diabetes in a synergistic manner with substantially no incidence of hepatotoxicity. Statins taken in combination with other drugs will cause a drug-drug interaction that will inhibit hepatic CYP-450. This class of enzymes is primarily responsible for the metabolism of drugs in the liver. P5P and related compounds are co-enzymes for many enzymes and do not inhibit these liver enzymes. Therefore, there are no adverse drug-drug interactions between statins and vitamin B6 related compounds. For example, an increase in the alanine transferase marker has been observed during statin therapy. This indicates potential hepatotoxicity. P5P and related compounds do not increase alanine transferase levels in the liver and therefore are not hepatotoxic.

[0035] Furthermore, the pharmaceutical compositions according to the present invention reduce the risk of cardiovascular disease and diabetes by positively influencing levels of alkaline phosphatase and PLA_2. The pharmaceutical compositions according to the present invention can also be used to reduce the risk of Alzheimer's disease and osteoporosis.

[0036] With respect to alkaline phosphatase, P5P and related compounds are natural substrates for this compound. Alkaline phosphatase is implicated in bone mineralization. The link between P5P and alkaline phosphatase has been particularly document in the study of hypophosphatasia. Low serum levels of alkaline phosphatase and a range of skeletal deformities characterize hypophosphatasia. Increasing levels of P5P will improve this disorder. In contrast, studies have shown that plasma levels of bone turnover markers including alkaline phosphatase were lower in statin treated subjects than in control subjects. Thus, in combination, statins and vitamin B6 related compounds beneficially regulate bone turnover.

[0037] With respect to secretory phospholipase A2 (PLA_2), in addition to lipid lowering properties, statins have been shown to reduce PLA_2 levels (Wiklund et al., Effects of Simstatin and atorvastin on inflammation marker in plasma, J. Intern Med., 2002, 251:338-347). PLA_2 has been indicated as is a strong independent risk factor for coronary heart disease (Camejo et al, Phospholipase A_2 in Vascular Disease, Circ Res. 2001, 89:298:304 at 298) and is also considered an inflammatory biomarker. PLA_2 catalyses the hydrolysis of the sn-2 ester bond in glyceroacyl phospholipids present in lipoproteins and cell membranes forming non-esterified fatty acids and lysophopholipids.

[0038] PLA_2 plays a role in several processes which increase the risk for cardiovascular disease. PLA_2 can modify circulating lipoproteins and induce the formation of LDL particles associated with increased risk for cardiovascular disease (Camejo et al., 2001, at p. 298). In arterial walls, PLA_2 can induce aggregation and

fusion of matrix-bound lipoproteins and further increase their binding strength to matrix proteoglycans. PLA_2 catalyzes the release of arachidonic acid from cell membranes which is converted by cycloxygenases to thromboxanes which promote vasoconstriction and platelet adhesion. Arachidonic acid is also converted by cycloxygenases to prostaglandins which mediate inflammation, a further cardiovascular disease risk factor. Prostaglandins and other inflammatory mediators influence multiple processes, including cholesterol homeostasis and coagulation.

[0039] P5P, a vitamin B6 metabolite has been implicated in the inhibition of arachidonic acid release via PLA_2 activation (Krinshnamurthi and Kakkar, Effect of pyridoxal 5'phosphate (PALP) on human platelet aggregation, dense granule release and thromboxane B2 generation – role of Schiff base formation, Thromb Haemost. 1982, 48:136). Thus, in combination, statins and vitamin B6 related compounds beneficially regulate PLA_2 levels.

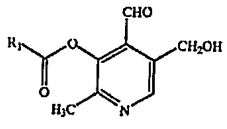
[0040] The present inventors are the first to employ a vitamin B6 related compound as an active agent for the reduction of cholesterol and PLA_2 in combination with a statin. The present inventors have discovered that the lipid lowering and PLA_2 inhibition properties of vitamin B6 related compounds are significantly greater than those for vitamin B6 and other previously disclosed vitamin B6 derivatives (see US Patent 6,066,659 and German patent DE 24 61 742 C2). P5P is forty times more potent in vivo as compared to pyroxidine. The inventors have also discovered that cardiovascular protective effects of statin and vitamin B6 related compounds are synergized when they are administered in combination. The inventors have further discovered that the statins and vitamin B6 related compounds do not react adversely when coadministered. Vitamin B6 related compounds do not inhibit hepatic CYP enzymes and do not increase hepatic transaminases. Accordingly, the pharmaceutical compounds of the present invention are non-hepatotoxic.

[0041] In light of these discoveries, the present invention provides pharmaceutical compositions and uses thereof for reducing the risk of cardiovascular disease and diabetes. The pharmaceutical compositions of the present invention are more effective than currently available combination therapies in reducing risk of cardiovascular disease. The pharmaceutical compositions ameliorate multiple risk factors including lipoproteins, homocysteine, vasoconstriction, platelet aggregation and inflammation. Furthermore, the pharmaceutical compositions do not induce hepatotoxicity. The pharmaceutical compositions of the present invention are comprised of a HMG CoA reductase inhibitor, a vitamin B6 related compound or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

[0042] Examples of HMG CoA reductase inhibitors that may be used include but are not limited to pravastatin (Pravachol™), lovastatin (Mevacor™), fluvastatin (Lescol™), atorvastatin (Lipitor™), simvastatin (Zocor™), rosuvastatin (Crestor™), velostatin, and fluindostatin. Preferably, the HMG CoA reducatase inhibitor is simvastatin. The term "HMG CoA reductase inhibitor" is intended to include all pharmaceutically acceptable salt, ester, and lactone forms of compounds that have HMG CoA reductase inhibitory activity.

[0043] Examples of the vitamin B6 related compound which may be used include but are not limited to pyridoxal-5-phosphate (P5P), pyridoxal, and pyridoxamine. Other vitamin B6 related compounds, which can also be used, include the 3-acylated analogues of pyridoxal, 3'acylated analogues of pyridoxal-4,5-aminal, and pyridoxine phosphonate analogues as disclosed in US Patent No. 6,585,414 and U.S. Patent Application No. 20030114424, both of which are incorporated herein by reference. Preferably, the vitamin B6 related compound will be P5P.

[0044] The 3-acylated analogue of pyridoxal includes:



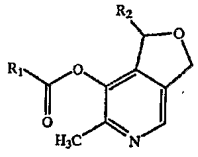
wherein,

R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy.

[0045] The 3-acylated analogue of pyridoxal-4,5-aminal includes:



wherein,

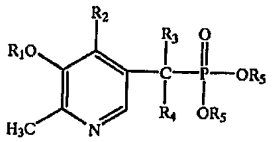
R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

R_2 is a secondary amino group.

[0046] The pyridoxine phosphate analogue includes:

(a) 

wherein,

R_1 is hydrogen or alkyl;

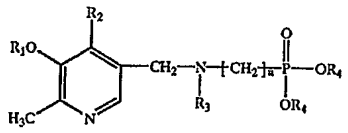
R_2 is –CHO-, -CH_2OH, -CH_3, -CO_2R6 in which R6 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, alkanoyloxy, alkylamino, or arylamino; or

R_3 and R_4 are halo; and

R_5 is hydrogen, alkyl, aryl, aralkyl, or –CO_2R_7 in which R_7 is hydrogen, alkyl, aryl, or aralkyl;

(b) 

wherein,

R_1 is hydrogen or alkyl;

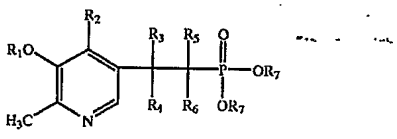
R_2 is –CHO, -CH_2OH, -CH_3, -CO_2R_5 in which R_5 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen, alkyl, aryl, aralkyl,

R_4 is hydrogen, alkyl, aryl, aralkyl, or -CO_2R6 in which R6 is hydrogen, alkyl, aryl or aralkyl;

n is 1 to 6; and

(c) 

wherein,

R_1 is hydrogen or alkyl;

R_2 is –CHO-, CH_2OH-, -CH_3, -CO_2R_8 in which R_8 is hydrogen, alkyl, aryl; or

R_2 is $–CH_2$-O alkyl- in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, or alkanoyloxy; or

R_3 and R_4 can be taken together to form =O;

R_5 and R6 are hydrogen; or

R_5 and R6 are halo;

R_7 is hydrogen, alkyl, aryl, aralkyl, or $–CO_2R_8$ in which R_8 is hydrogen, alkyl, aryl, or aralkyl.

[0047] It is to be understood that this invention is not limited to specific dosage forms, carriers, or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[0048] Some of the compounds described herein contain one or more asymmetric centres and this may give raise to enantiomers, disasteriomers, and other stereroisomeric forms which may be defined in terms of absolute stereochemistry as (R)- or (S)-. The present invention is meant to include all such possible diasteriomers and enantiomers as well as their racemic and optically pure forms. Optically active (R)- and (S)- isomers may be prepared using chiral synthons or chiral reagents, or resolved using conventional techniques. When the compounds described herein contain olefinic double bonds or other centres of geometric symmetry, and unless specified otherwise, it is intended that the compounds include both E and A geometric isomers. Likewise all tautomeric forms are intended to be included.

[0049] As used in this specification and the appended claims, the singular forms "a," "an", and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an active agent" or "a pharmacologically active agent" includes a single active agent as well as two or more different active agents in combination, reference to "a carrier" includes mixtures of two or more carriers as well as a single carrier, and the like.

[0050] By "pharmaceutically acceptable," such as in the recitation of a "pharmaceutically acceptable carrier," or a "pharmaceutically acceptable salt," is meant herein a material that is not biologically or otherwise undesirable, i.e., the material may be incorporated into a pharmaceutical composition administered to a patient without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the composition in which it is contained.

[0051] "Carriers" or "vehicles" as used herein refer to conventional pharmaceutically acceptable carrier materials suitable for drug administration, and include any such materials known in the art that are nontoxic and do not interact with other components of a pharmaceutical composition or drug delivery system in a deleterious manner.

[0052] By an "effective" amount or a "therapeutically effective amount" of a drug or pharmacologically active agent is meant a nontoxic but sufficient amount of the drug or agent to provide the desired effect. In the combination therapy of the present invention, an "effective amount" of one component of the combination is the amount of that compound that is effective to provide the desired effect when used in combination with the other components of the combination. The amount that is "effective" will vary from subject to subject, depending on the age and general condition of the individual, the particular active agent or agents, and the like. Thus, it is not always possible to specify an exact "effective amount." However, an

appropriate "effective" amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

[0053] The terms "reduce the risk of cardiovascular disease" and "reducing the risk of cardiovascular disease" as used herein refer to the reduction or elimination of an underlying cause or biomarker associated with the increased incidence of a cardiovascular event.

[0054] As used herein, "cardiovascular disease" means any disease of the heart of blood vessels. Examples of cardiovascular disease include: congestive heart failure, myocardial ischemia, arrhythmia, myocardial infarction, ischemic stroke, hemorrhagic stroke, coronary artery disease, hypertension (high blood pressure), atherosclerosis (clogging of the arteries), aneurysm, peripheral artery disease (PAD), thrombophlebitis (vein inflammation), diseases of the heart lining, diseases of the heart muscle, carditis, congestive heart failure, endocarditis, ischemic heart disease, valvular heart disease (malfunction of a valve or valves in the blood vessels of the heart), arteriosclerosis (hardening of the arteries), acute coronary syndrome (ACS), high cholesterol, deep vein thrombosis (DVT), Kawazaki disease, and heart transplant.

[0055] The terms "reduce the risk of diabetes" and "reducing the risk of diabetes" as used herein refer to the reduction or elimination of an underlying cause or biomarker associated with the increased incidence of developing insulin resistance, pre-diabetes and diabetes.

[0056] As used herein, "vitamin B6 related compound", means any vitamin B6 precursor, metabolite, derivative, or analogue thereof but explicitly excludes: (1) vitamin B6 (pyroxidine); (2) the 5' phosphoric acid esters of pyridoxal, pyridoxol and pyridoxamine disclosed in German Patent DE 24 61 742 C2, and (3) the pyridoxine, pyridoxal, and pyridoxamine derivatives disclosed in US Patent No. 6,066,659.

[0057] As used herein, "hepatotoxicity" includes any drug-induced liver injury.

[0058] The pharmaceutical compositions of the present invention may be manufactured in a manner that is itself known, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0059] Pharmaceutical compositions for use in accordance with the present invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

[0060] For injection, the agents of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiological saline buffer.

[0061] For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a patient to be treated. Pharmaceutical preparations for oral use can be obtained by solid excipient, optionally grinding a resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol, or cellulose preparations such as, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and/or polyvinylpyrrolidone. If desired, disintegrating agents may be added, such as the

cross-linked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0062] Preferably, the pharmaceutical compositions of the present invention are administered orally. Preferred oral dosage forms contain a therapeutically effective unit dose of each active agent, wherein the unit dose is suitable for a once-daily oral administration. The therapeutic effective unit does of any of the active agents will depend on number of factors which will be apparent to those skilled in the art and in light of the disclosure herein. In particular these factors include: the identity of the compounds to be administered, the formulation, the route of administration employed, the patient's gender, age, and weight, and the severity of the condition being treated and the presence of concurrent illness affecting the gastro-intestinal tract, the hepatobillary system and the renal system. Methods for determining dosage and toxicity are well known in the art with studies generally beginning in animals and then in humans if no significant animal toxicity is observed. The appropriateness of the dosage can be assessed by monitoring LDL levels, HDL levels, total cholesterol levels, triglycerides levels, and homocysteine levels. Where the dose provided does not cause LDL lipoprotein and homocysteine levels to decline to normal or tolerable levels, following at least 2 to 4 weeks of treatment, the dose can be increased.

[0063] The therapeutic effective unit dosage for the HMG CoA reductase inhibitor is between 0.1 mg and 1000 mg per day. Suitable dosage ranges for particular HMG CoA reductase inhibitors are known in the art. Typically the unit dosage will be between 5, 10, 20, 40, and 80 mg per day. Where the HMG CoA reductase inhibitor employed is simvastin, the preferred unit dosage is 10 mg per day. The preferred unit dosage for other HMG CoA reductase inhibitors is 20 mg per day.

[0064] The preferred therapeutic effective unit dosage for the vitamin B6 related compound is between 0.1 to 50 mg/kg body weight daily. More preferably, the unit dosage will be 1 to 15 mg/kg body weight daily.

[0065] The present invention also provides a method of treating or preventing hypercholesterolemia in a patient comprising administering a therapeutically effective dose of a vitamin B6 related compound wherein the vitamin B6 related compound is pyridoxal-5'-phosphate, a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, or a mixture thereof.

Example One – Effect of P5P on CYP Activity

[0066] The inhibitory effect of P5P on the activity of hepatic cytochrome enzymes was examined *in vitro*. The CYP inhibition assays used microsomes (Supersomes®, Gentest Corp., Woburn, MA) prepared from insect cells, each expressing an individual CYP subtype (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4) expressed from the corresponding human CYP cDNA using a baculovirus expression vector. The microsomes also incorporated supplemental cDNA-expressed human reductase and/or cytochrome b5, as these enzymes stimulate the activity of the CYPs, allowing for a reduction in the amount of enzyme required per reaction (Gentest Corp.). The assays monitored, *via* fluorescence detection, the formation of a fluorescent metabolite following incubation of the microsomes with a specific CYP substrate. Two CYP substrates (7-benzyloxy-4-trifluoromethylcoumarin (BFC) and 7-benzyloxycoumarin (BQ)) were tested for CYP3A4, as this enzyme has been shown to exhibit complex inhibition kinetics. Reactions (0.2 mL) were performed in 96-well microtitre plates at 37°C in the presence of an NADPH regenerating system [NADP+, glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PDH)] and $MgCl_2$. Inhibition of metabolic product formation by pyridoxal 5'-phosphate for each enzyme was tested in the absence (0 µM) and presence of 0.0169 to 37.0 µM pyridoxal 5'-phosphate. An enzyme-selective inhibitor was also tested at 8 concentrations in each assay as a positive control. All determinations were performed in duplicate. The reagent solutions used for all of the CYP subtype assays, except CYP2C19 and CYP3A4, were prepared by MBDI. For CYP2C19 and CYP3A4, complete reagent kits

purchased from Gentest Corp. (CYP2C19/CEC: Cat. No. HTS-4000, Lot No. 1; CYP3A4/BFC: Cat. No. HTS-1000, Lot No. 1) were used to perform the assays.

[0067] Assays for all enzymes were performed in the following manner: the NADPH regenerating system, appropriate buffer solution and vehicle, inhibitor (positive control) solution or test compound (pyridoxal 5'-phosphate) solution were dispensed into 96-well microtitre plates. Eight inhibitor and test compound concentrations were tested using 3-fold serial dilutions. The microtitre plates containing 0.1 mL/well of the latter mixture were pre-warmed to 37°C in an incubator. A solution of buffer, microsomes and substrate was separately prepared and vortex mixed to disperse the protein. The reactions were initiated by the addition of the microsome/substrate solution (0.1 mL) to the wells of the microtitre plates containing the pre-warmed NADPH regenerating system, buffer and inhibitor solutions. Following specified incubation times, the reactions were stopped by the addition of 0.075 mL of a STOP solution (see below). Blank (background noise) samples were also assayed by adding the STOP solution prior to the addition of the microsome/substrate mix to the NADPH regenerating system. The amount of metabolic product formed was quantified by fluorescence detection in a fluorescence plate reader utilizing excitation and emission filters that had been optimized for the detection of each metabolite.

[0068] Prior to performing the CYP inhibition assays, the effect of pyridoxal 5'-phosphate on the fluorescence of the metabolic products measured in the assays was evaluated. The fluorescence of metabolite (one concentration, in duplicate) was measured in the absence (0 µM) and presence of 0.457 to 1000 µM pyridoxal 5'-phosphate. The concentrations and metabolic products measured were: 1 µM 3-cyano-7-hydroxycoumarin (CHC), 2.5 µM 7-hydroxycoumarin (7-HC), 2.5 µM 7-hydroxy-4-trifluoromethylcoumarin (HFC), 0.1 µM fluorescein, 10 µM 3-[2-(N,N-diethylamino)ethyl]-7-hydroxy-4-methylcoumarin (AHMC) and 10 µM quinolinol. The concentration of metabolite used was based on the expected maximum

concentration of metabolite formed in the CYP inhibition assay (*i.e.* the concentration of metabolite measured following incubation substrate with the CYP subtype in the absence of an inhibitor). CHC is the fluorescent metabolite measured in the CYP1A2 and CYP2C19 assays. 7-HC is the fluorescent metabolite measured in the CYP2A6 assay, HFC is the fluorescent metabolite measured in the CYP2B6, CYP2C9, CYP2E1 and CYP3A4 (BFC as substrate) assays and fluorescein is the metabolite measured in the CYP2C8 assay. AHMC is the metabolite measured in the CYP2D6 assay and quinolinol is measured in the CYP3A4 (BQ as substrate) assay.

[0069] Pyridoxal 5'-Phosphate Solution - pyridoxal 5'-phosphate monohydrate (P5P, Lot No. 00001448) was supplied as powder. The concentrations of all pyridoxal 5'-phosphate solutions are based on the anhydrous molecular weight (247.15 g/mole) corrected for a potency factor of 0.9019.

[0070] For the determination of the effect of pyridoxal 5'-phosphate on metabolite fluorescence, a stock solution of pyridoxal 5'-phosphate, at a concentration of 50 mM, was freshly prepared in distilled water. Since pyridoxal 5'-phosphate is acidic in aqueous solution, the pH of the solution was adjusted to 7.0 with 1 N NaOH. The solution of pyridoxal 5'-phosphate was added to the wells of the microtitre plate starting with a 50-fold dilution to 1000 µM, followed by 3-fold serial dilutions to: 333, 111, 37.0, 12.3, 4.12, 1.37 and 0.457 µM.

[0071] For the CYP subtype inhibition assays, a stock solution of pyridoxal 5'-phosphate, at a concentration of 50 mM, was freshly prepared in distilled water (pH adjusted to 7.0 with 1 N NaOH). The solution of pyridoxal 5'-phosphate was diluted with distilled water to 111 µM and then added to the wells of the microtitre plate starting with a 3-fold dilution to 37.0 µM, followed by 3-fold serial dilutions to: 12.4, 4.12, 1.37, 0.457, 0.152, 0.0508 and 0.0169 µM.

[0072] Data Analysis - The mean of the duplicate fluorescent signals in the presence and absence (vehicle control) of each compound was calculated and corrected for the background noise. Percent inhibition was calculated as the difference in the corrected fluorescent signals in the absence and presence of the compound, divided by the corrected fluorescent signal in the absence of compound, multiplied by 100%. The concentration of the inhibitor or pyridoxal 5'-phosphate, where appropriate, which inhibited metabolite formation by 50% (IC_{50}) was calculated by nonlinear regression analysis (sigmoidal dose-response curve) of the % Inhibition *versus* Log concentration data using GraphPad Prism software (Version 3.00, GraphPad Software, Inc., San Diego, CA).

[0073] RESULTS - The effect of pyridoxal 5'-phosphate on the fluorescence of the various metabolic products measured in the CYP inhibition assays was determined. As evident in Figure 1, pyridoxal 5'-phosphate significantly quenched (decreased) the fluorescence of five the six metabolites measured in the assays (CHC, 7-HC, 7-HFC, AHMC and quinolinol) at concentrations of > 37 µM. Pyridoxal 5'-phosphate (up to 1000 µM) did not affect the fluorescence of fluorescein, the metabolic product measured following the metabolism of dibenzylfluorescein by the CYP2C8 enzyme. The inhibitory effect of pyridoxal 5'-phosphate on CYP catalytic activity was tested over the concentration range of 0.0169 to 37 µM.

[0074] The results from the incubation of the known inhibitors and pyridoxal 5'-phosphate with each of the CYP subtypes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4) are depicted graphically in Figures 2 to 11. The observed IC_{50} values for the various CYP inhibitors are similar to those obtained previously in our laboratory during assay validation (for CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP2E1) and are similar to those determined by the supplier (for the CYP2C19 and CYP3A4 assay kits) (Figure 12). These data indicate that enzyme activity was not compromised in any of the assays.

[0075] Over the concentration range tested (0.0169 to 37.0 μM), pyridoxal 5'-phosphate did not inhibit the catalytic acitivity of seven of the CYP enzymes: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP2E1 (Figures 2, 3, 4, 5, 6, 8 and 9, respectively). Pyridoxal 5'-phosphate did, however, inhibit the metabolic activity of the CYP2C19 and CYP3A4 enzyme subtypes (Figures 7, 10 and 11). The potency of pyridoxal 5'-phosphate was relatively similar for the CYP2C19 and CYP3A4 enzyme subtypes (IC_{50} values of ~33 and ~37 μM, respectively). Pyridoxal 5'-phosphate appeared to inhibit the CYP3A4 enzyme-mediated metabolism of the substrate BFC to a slightly greater extent ($IC_{50} \cong 37$ μM) than the substrate BQ (IC_{50} >37 μM, Figures 10 and 11, respectively). A summary of the IC_{50} values for pyridoxal 5'-phosphate and the known inhibitors is given in Figure 12.

[0076] CONCLUSIONS - The compound pyridoxal 5'-phosphate did not selectively inhibit the catalytic activity of seven CYP subtypes: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2D6 and CYP2E1, over the concentration range tested (0.0169 to 37.0 μM). Clinically relevant drug interactions would, therefore, not be expected to occur between pyridoxal 5'-phosphate and substrates of these enzymes. Pyridoxal 5'-phosphate did selectively inhibit the catalytic activity of two (CYP2C19 and CYP3A4) of the nine human CYP subtypes tested at relatively high concentrations (IC_{50} = 33 μM for CYP2C19 and ≥37 μM for CYP3A4). However, based on the relatively low inhibitory potency of pyridoxal 5'-phosphate for the two CYP subtypes *in vitro*, the occurrence of serious drug interactions is expected to be unlikely.

Example Two – Human Clinical Study

[0077] A double blinded, placebo-controlled study of 300 subjects (18 years or older) at high risk for cardiovascular disease is conducted. Risk factors included: smoking, menopause, hypertension, prior heart attacks, angina, hypercholesterolemia, hyperhomocysteinemia, obesity and diabetes.

[0078] The subjects are placed into three groups. Group One is given a placebo pill. Group Two is given a HMG CoA reductase inhibitor selected from the group consisting of pravastatin, lovastatin, fluvastatin, atorvastatin, simvastatin, rosuvastatin, velostatin, and fluindostatin and Group Three is given the combination tablet having the following formulation wherein the HMG CoA reductase inhibitor is also selected from the group consisting of pravastatin, lovastatin, fluvastatin, atorvastatin, simvastatin, rosuvastatin, velostatin, and fluindostatin:

Tablet Ingredient	Percentage by Weight	Actual Weight
P5P	66.25	265.00 mg
Statin	5	20.00 mg
Povidone K30 USP	4.687	18.75 mg
Microcrystalline cellulose 102	17.125	68.500 mg
Croscarmellose cellulose	3.00	12.00 mg
Talc	2.250	9.00 mg
Silicon dioxide	0.562	2.25 mg
Magnesium stearate	1.125	4.50 mg
	Total Weight	400.00 mg

[0079] People ineligible for the study met one or more of the exclusion criteria:

- Pregnancy or potential for pregnancy;
- History of alcohol or drug abuse within the past year;
- Participation in any other investigational drug study within 30 days of randomization;
- Any medical or psychiatric condition which in the opinion of the investigator makes the patient an unsuitable candidate for the study;
- Severe renal dysfunction defined as a serum creatinine value = 250 micromol/L (2.8 mg/dL) or nephrotic syndrome at screening;
- History of liver cirrhosis, chronic active hepatitis or severe liver dysfunction, or liver transaminase =3 times ULN at screening;
- Intolerance to any of the cholesterol lowering drugs; and

- Uncontrolled diabetes defined as plasma blood glucose value equal to or greater than 24 mmol/L at the time of screening.

[0080] The study lasts one year and participants receive the following tests at baseline, then again every week for the first month and once a month for the remaining 11 months: total LDL levels; total HDL levels; triglyceride levels; plasma total homocysteine; alkaline phosphatase; alanine transferase; CYP activity; blood pressure; total bilirubin; bun; creatinine; potassium; liver transaminase; percentage total cholesterol/LDL; and % total cholesterol/HDL.

[0081] The results of the study show that the subjects treated with P5P and the HMG Co reductase inhibitor have lower LDL and triglyceride levels, higher HDL levels lower homocysteine levels, no hepatotoxic effects and in general are in better cardiovascular health.

[0082] Although the present invention has been described with reference to illustrative embodiments, it is to be understood that the invention is not limited to these precise embodiments, and that various changes and modifications may be effected therein by one skilled in the art. All such changes and modifications are intention to be encompassed in the appended claims.

CLAIMS

What is claimed is:

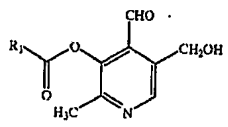
1. A pharmaceutical composition comprising: (a) a HMG CoA reductase inhibitor; (b) a vitamin B6 related compound; and (c) a pharmaceutically acceptable carrier.

2. The pharmaceutical composition according to claim 1 wherein the HMG CoA reductase inhibitor is selected from a group consisting: pravatstatin, lovastatin, fluvastatin, atorvastatin, simvastatin, rosuvastatin, velostatin, fluindostatin, and a mixture thereof.

3. The pharmaceutical composition according to claim 1 wherein the vitamin B6 related compound is selected from a group consisting: pyridoxal, pyridoxal-5'-phosphate, pyridoxamine, a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, and a mixture thereof.

4. The pharmaceutical composition according to claim 1 wherein the vitamin B6 related compound is pyridoxal-5-phosphate.

5. The pharmaceutical composition according to claim 3 wherein the 3-acylated analogue of pyridoxal is:



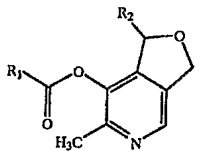
wherein,

R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

6. The pharmaceutical composition according to claim 3 wherein the 3-acylated analogue of pyridoxal-4,5-aminal is



wherein,

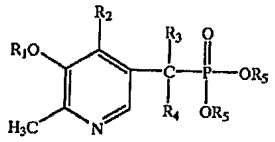
R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

R_2 is a secondary amino group.

7. The pharmaceutical composition according to claim 3 wherein the pyridoxine phosphate analogue is selected from a group consisting:



(a)

wherein,

R_1 is hydrogen or alkyl;

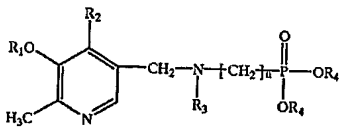
R_2 is –CHO-, -CH_2OH, -CH_3, -CO_2R6 in which R6 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, alkanoyloxy, alkylamino, or arylamino; or

R_3 and R_4 are halo; and

R_5 is hydrogen, alkyl, aryl, aralkyl, or –CO_2R_7 in which R_7 is hydrogen, alkyl, aryl, or aralkyl;



(b)

wherein,

R_1 is hydrogen or alkyl;

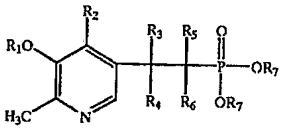
R_2 is –CHO, -CH_2OH, -CH_3, -CO_2R_5 in which R_5 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen, alkyl, aryl, aralkyl,

R_4 is hydrogen, alkyl, aryl, aralkyl, or -CO_2R6 in which R6 is hydrogen, alkyl, aryl or aralkyl;

n is 1 to 6; and

(c) 

wherein,

R_1 is hydrogen or alkyl;

R_2 is –CHO-, CH_2OH-, -CH_3, -CO_2R_8 in which R_8 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl- in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, or alkanoyloxy; or

R_3 and R_4 can be taken together to form =O;

R_5 and R6 are hydrogen; or

R_5 and R6 are halo;

R_7 is hydrogen, alkyl, aryl, aralkyl, or $-CO_2R_8$ in which R_8 is hydrogen, alkyl, aryl, or aralkyl.

8. A method for treating a patient at risk of cardiovascular disease comprising administering a therapeutically effective dose of the pharmaceutical composition according to any one of claims 1 to 7.

9. The method according to claim 8, wherein the patient is susceptible to hepatotoxicity.

10. The method according to claim 8 wherein the cardiovascular disease is selected from a group consisting: congestive heart failure, myocardial ischemia, arrhythmia, myocardial infarction, ischemic stroke, hemorrhagic stroke, coronary artery disease, hypertension (high blood pressure), atherosclerosis (clogging of the arteries), aneurysm, peripheral artery disease (PAD), thrombophlebitis (vein inflammation), diseases of the heart lining, diseases of the heart muscle, carditis, congestive heart failure, endocarditis, ischemic heart disease, valvular heart disease (malfunction of a valve or valves in the blood vessels of the heart), arteriosclerosis (hardening of the arteries), acute coronary syndrome (ACS), deep vein thrombosis (DVT), Kawazaki disease, high cholesterol, and heart transplant.

11. The method according to claim 8 wherein the dose of the HMG CoA reductase inhibitor is between 0.1 and 1000 mg per day.

12. The method according to claim 8 wherein the dose of the HMG CoA reductase inhibitor is 10 mg per day.

13. The method according to claim 8 wherein the dose of the HMG CoA reductase inhibitor is 20 mg per day.

14. The method according to claim 8 wherein the dose of the vitamin B6 related compound is between 0.1 to 50 mg/kg per day.

15. The method according to claim 8 wherein the dose of vitamin B6 related compound is between 1 to 15 mg/kg per day.

16. A method of a patient at risk for diabetes comprising administering a therapeutically effective dose of the pharmaceutical composition according to any one of claims 1 to 8.

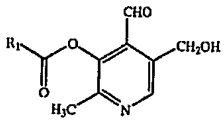
17. A method for treating a patient at risk of Alzheimer's disease comprising administering a therapeutically effective dose of the pharmaceutical composition according to any one of claims 1 to 7.

18. A method for treating a patient at risk of osteoporosis comprising administering a therapeutically effective dose of the pharmaceutical composition according to any one of claims 1 to 7.

19. A method of treating or preventing hypercholesterolemia in a patient, comprising administering a therapeutically effective dose of a vitamin B6 related compound wherein the vitamin B6 related compound is selected from a group consisting: pyridoxal-5'-phosphate, a 3-acylated analogue of pyridoxal, a 3-acylated analogue of pyridoxal-4,5-aminal, a pyridoxine phosphate analogue, and a mixture thereof.

20. The method according to claim 19 wherein the vitamin B6 related compound is pyridoxal-5-phosphate.

21. The method according to claim 19 wherein the 3-acylated analogue of pyridoxal is:



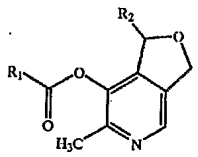
wherein,

R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

22. The method according to claim 16 or 17 wherein the 3-acylated analogue of pyridoxal-4,5-aminal is



wherein,

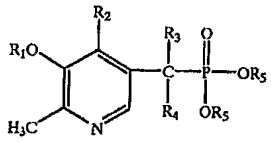
R_1 is alkyl, alkenyl, in which alkyl can interrupted by nitrogen, oxygen, or sulfur, and can be unsubstituted or substituted at the terminal carbon with hydroxy, alkoxy, alkanoyloxy, alkoxyalkanoyl, alkoxycarbonyl, or

R_1 is dialkylcarbamoyloxy; alkoxy; dialkylamino; alkanoyloxy; alkanoyloxyaryl; alkoxyalkanoyl; alkoxycarbonyl; dialkylcarbamoyloxy; or

R_1 is aryl, aryloxy, arylthio, or aralkyl, in which aryl can be substituted by alkyl, alkoxy, amino, hydroxy, halo, nitro, or alkanoyloxy;

R_2 is a secondary amino group.

23. The pharmaceutical composition according to claim 16 wherein the pyridoxine phosphate analogue is selected from a group consisting:

(a) 

wherein,

R_1 is hydrogen or alkyl;

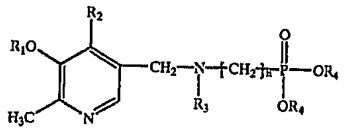
R_2 is –CHO-, -CH_2OH, -CH_3, -CO_2R6 in which R6 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, alkanoyloxy, alkylamino, or arylamino; or

R_3 and R_4 are halo; and

R_5 is hydrogen, alkyl, aryl, aralkyl, or –CO_2R_7 in which R_7 is hydrogen, alkyl, aryl, or aralkyl;

(b) 

wherein,

R_1 is hydrogen or alkyl;

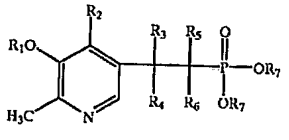
R_2 is –CHO, -CH_2OH, -CH_3, -CO_2R_5 in which R_5 is hydrogen, alkyl, aryl; or

R_2 is –CH_2-O alkyl in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen, alkyl, aryl, aralkyl,

R_4 is hydrogen, alkyl, aryl, aralkyl, or -CO_2R6 in which R6 is hydrogen, alkyl, aryl or aralkyl;

n is 1 to 6; and

(c) 

wherein,

R_1 is hydrogen or alkyl;

R_2 is –CHO-, CH_2OH-, -CH_3, -CO_2R_8 in which R_8 is hydrogen, alkyl, aryl; or

R_2 is $–CH_2$-O alkyl- in which alkyl is covalently bonded to the oxygen at the 3-position instead of R_1;

R_3 is hydrogen and R_4 is hydroxy, halo, alkoxy, or alkanoyloxy; or

R_3 and R_4 can be taken together to form =O;

R_5 and R6 are hydrogen; or

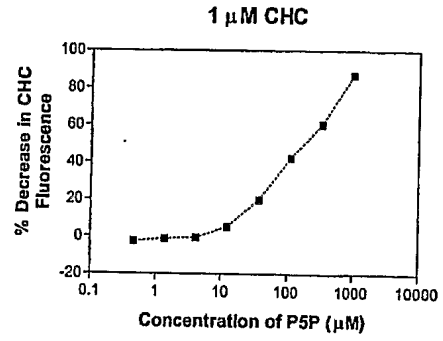
R_5 and R6 are halo;

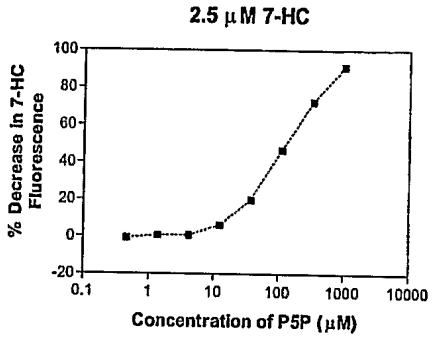
R_7 is hydrogen, alkyl, aryl, aralkyl, or $–CO_2R_8$ in which R_8 is hydrogen, alkyl, aryl, or aralkyl.

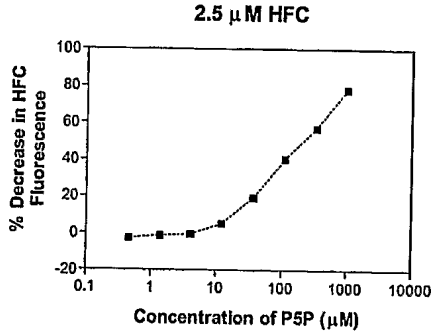
ABSTRACT

The present invention provides pharmaceutical compositions comprising a HMG CoA reductase inhibitor and a vitamin B6 related compound and methods for using the pharmaceutical compositions for reducing the risk of cardiovascular and other diseases.

FIGURE 1







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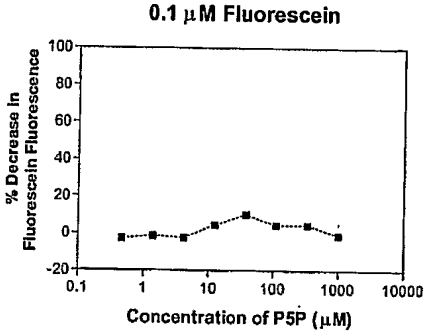

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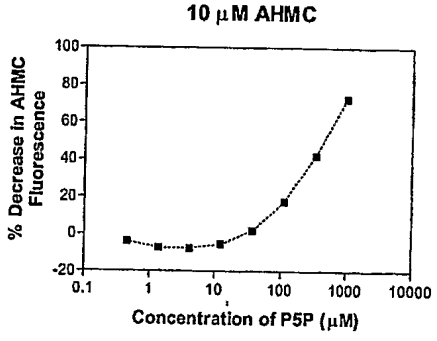


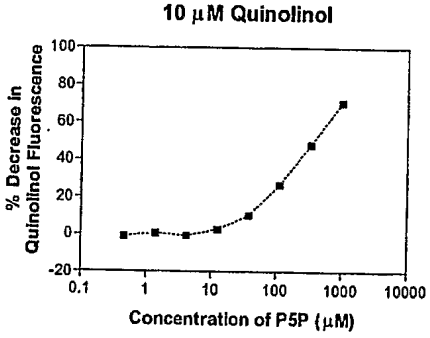
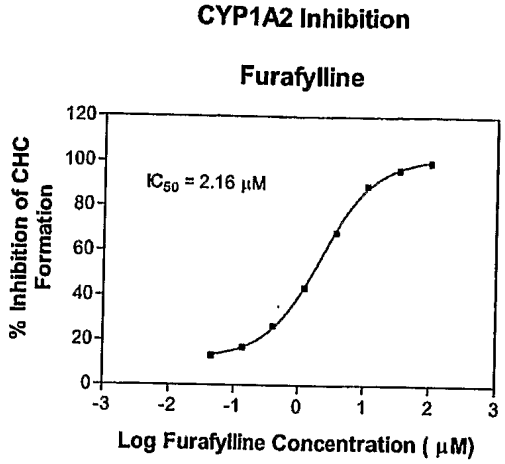


FIGURE 2



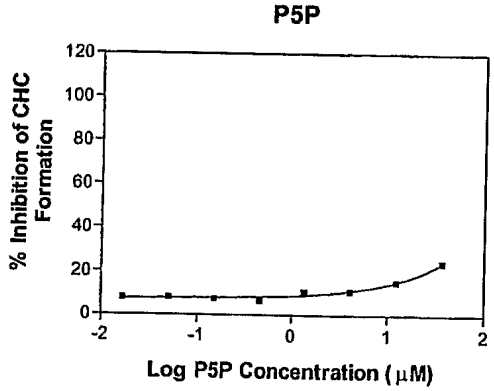
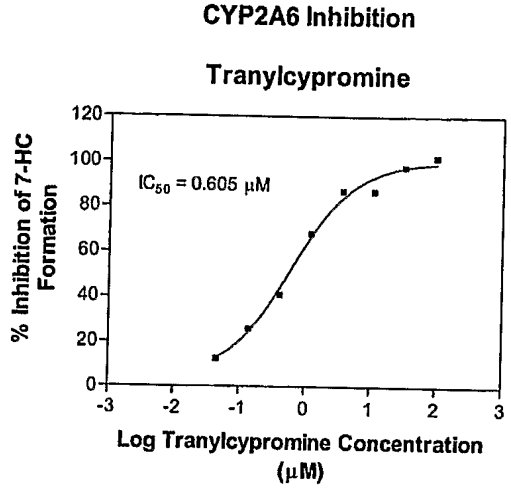


FIGURE 3



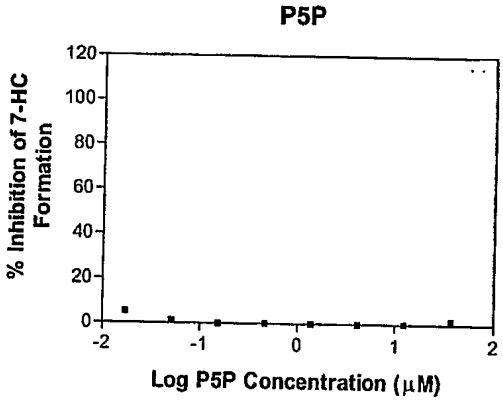
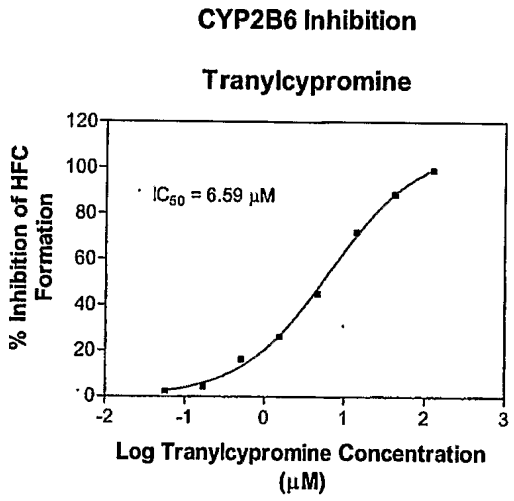


FIGURE 4



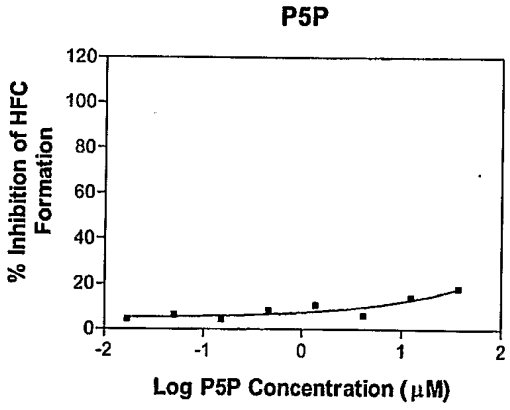
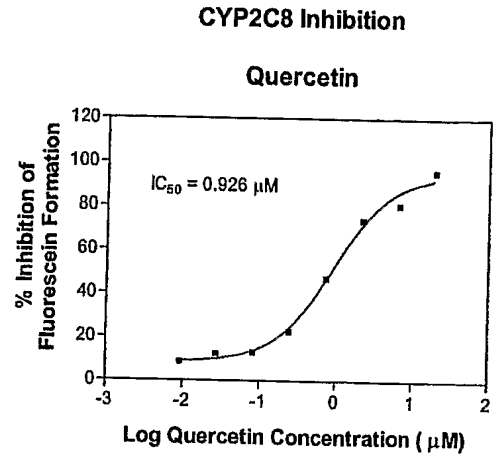


FIGURE 5



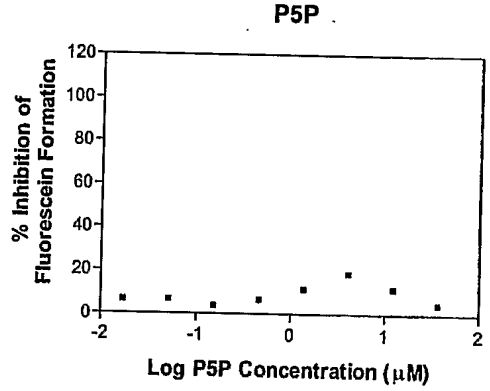
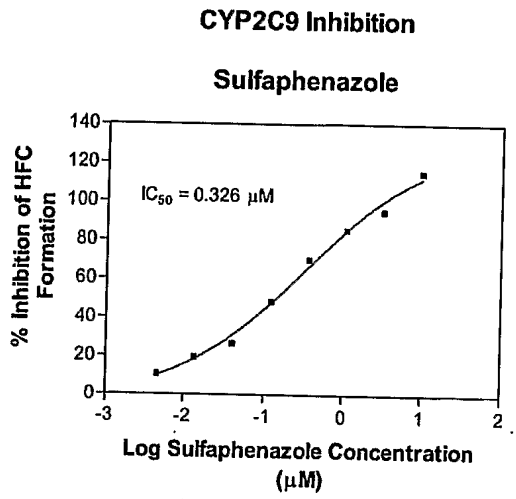


FIGURE 6



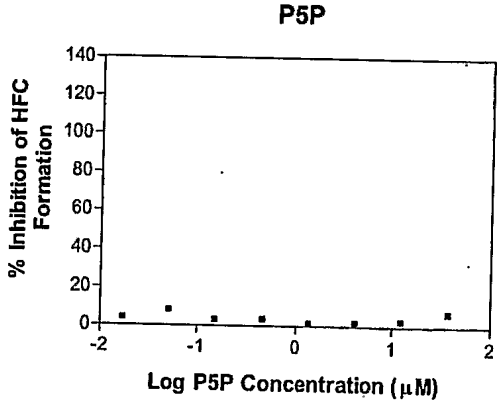
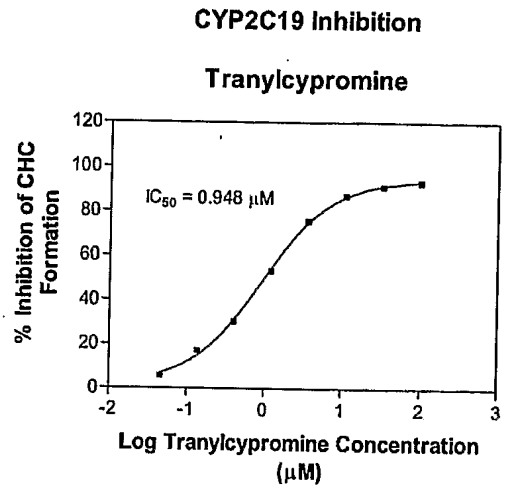


FIGURE 7



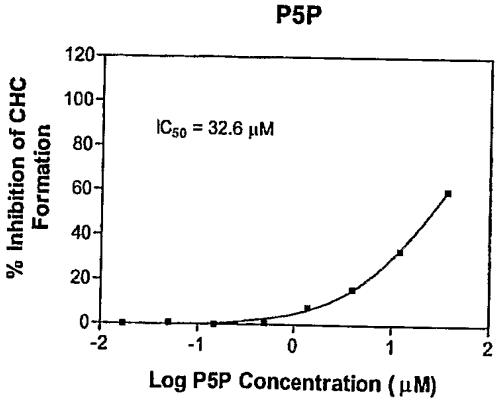
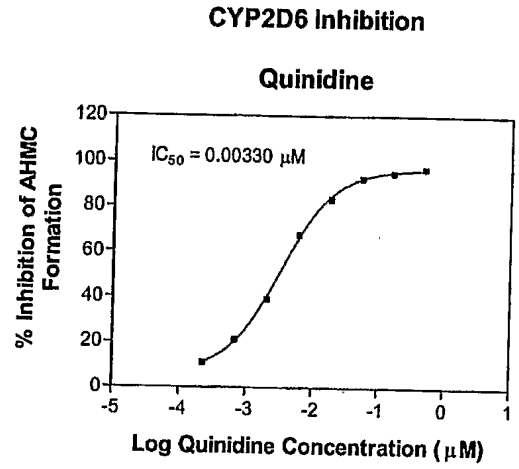


FIGURE 8



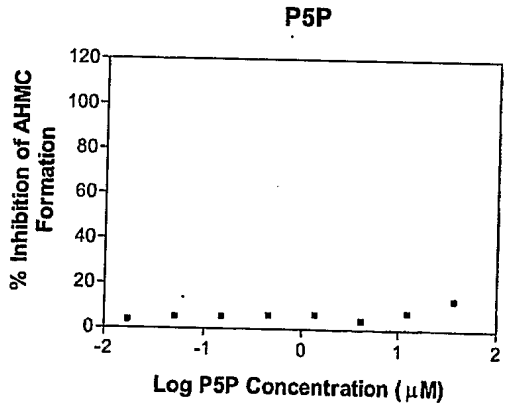
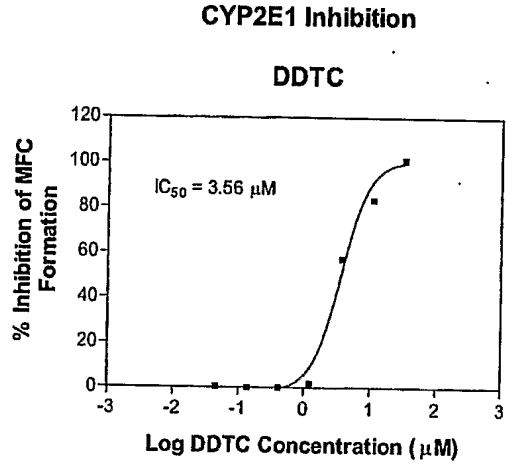


FIGURE 9



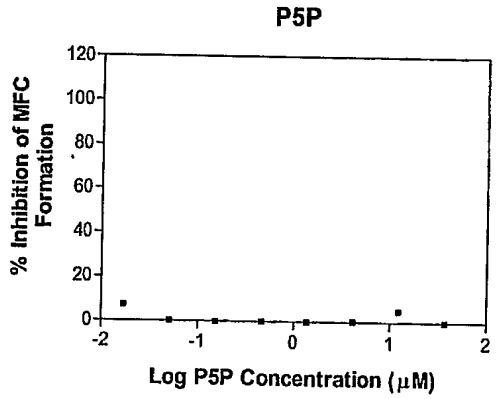


FIGURE 10

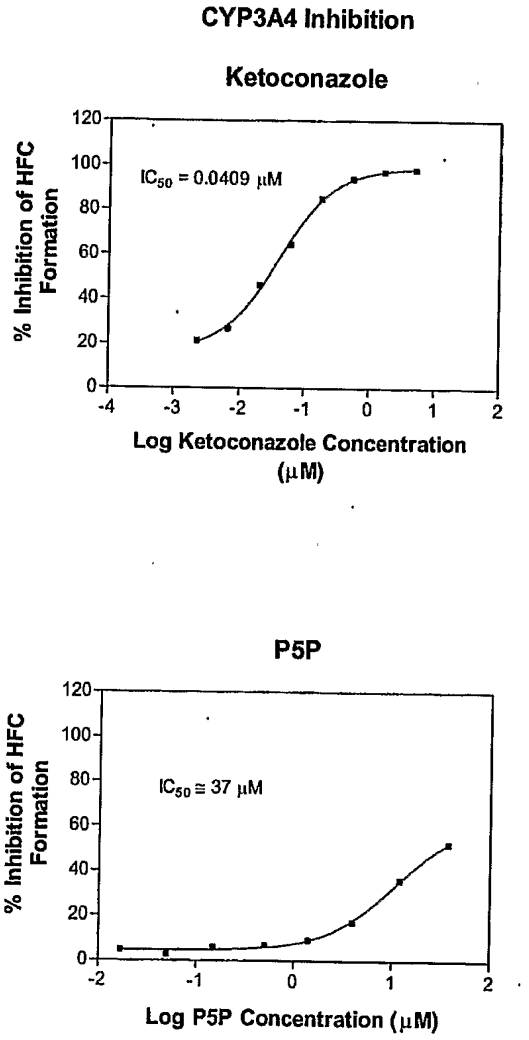
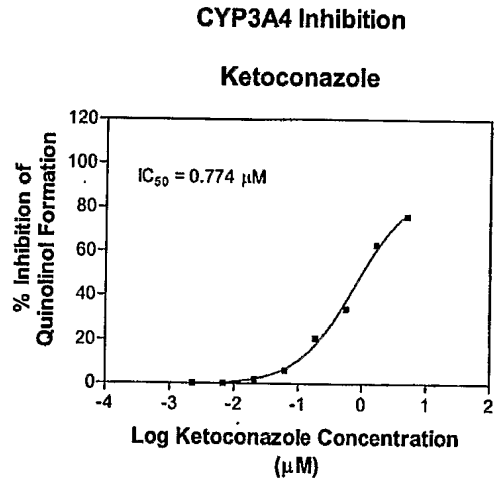


FIGURE 11



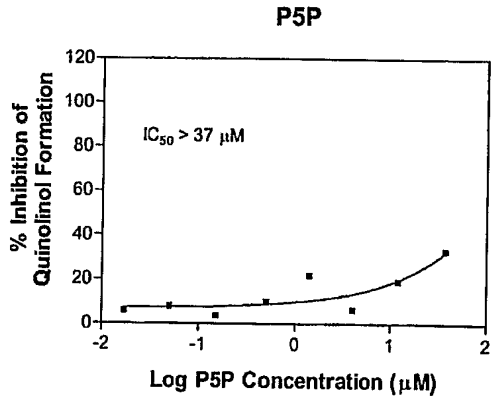


FIGURE 12

CYP Subtype	Substrate	Inhibitor (positive control)	Expected Inhibitor IC_{50}	Observed Inhibitor IC_{50}	Pyridoxal 5'-phosphate IC_{50}
			(μM)		
CYP1A2	CEC	Furafylline	2.22 ± 1.15[a]	2.16	na
CYP2A6	Coumarin	Tranylcypromine	0.990 ± 0.209[a]	0.605	na
CYP2B6	EFC	Tranylcypromine	8.82 ± 2.43[a]	6.59	na
CYP2C8	DBF	Quercetin	1.40 ± 0.240[a]	0.926	na
CYP2C9	MFC	Sulfaphenazole	0.401 ± 0.116[a]	0.326	na
CYP2C19	CEC	Tranylcypromine	0.825[b]	0.948	32.6
CYP2D6	AMMC	Quinidine	0.00544 ± 0.00173[a]	0.00330	na
CYP2E1	MFC	DDTC	5.06 ± 1.96[a]	3.56	na
CYP3A4	BFC	Ketoconazole	0.018[b]	0.0409	≅37
	BQ		0.400[b]	0.774	>37

[a] value expected from assay validation (mean ± S.D., n ≥8 experiments).
[b] value determined by the supplier using all the components contained in the current lot kit.
na denotes not applicable since concentration-dependent inhibition was not observed over the concentration range tested.